

ORIGINAL ARTICLE

Liver disease in transfusion dependent thalassaemia major

C K Li, K W Chik, C W K Lam, K F To, S C H Yu, V Lee, M M K Shing, A Y K Cheung, P M P Yuen

Arch Dis Child 2002;**86**:344–347

See end of article for authors' affiliations

Correspondence to: Dr C K Li, Department of Paediatrics, Prince of Wales Hospital, Shatin, Hong Kong SAR, China; ckli@cuhk.edu.hk

Accepted for publication 1 November 2001

Aims: To study the prevalence and severity of liver diseases of transfusion dependent thalassaemia major patients, and correlate the histological and biochemical changes of iron overload in liver with the peripheral blood markers.**Method:** Liver biopsy was performed to assess the histological changes and liver iron content (LIC).**Results:** One hundred patients were evaluated (median age 11.7 years, range 1.5–27). A total of 81 liver biopsies were performed in 73 patients; 43 samples were analysed for LIC. Grade 3–4 haemosiderosis and hepatic fibrosis was found in 44% and 30% of patients respectively; both were significantly associated with higher serum ferritin, liver enzymes, and LIC. Very high LIC (>15 mg/g dry weight) was present in 16.3% of patients.**Conclusion:** Severe haemosiderosis and hepatic fibrosis were common in patients with thalassaemia major despite the use of chelation therapy. Liver biopsy provided information on fibrosis and LIC which could not be accurately predicted from peripheral blood markers.

Thalassaemia major (TM) is the commonest hereditary form of transfusion dependent anaemia in Hong Kong and about 300 patients currently receive regular blood transfusion treatment.¹ With regular transfusion and iron chelation, most TM patients now survive beyond the third decade.² Although cardiomyopathy is still the leading cause of death in this group of patients, liver disease is becoming a more important cause of mortality.³ Cirrhosis and hepatocellular carcinoma may develop as result of chronic hepatitis and/or severe iron overload. Iron chelation with desferrioxamine can reduce excessive body iron, but efficacy is limited by poor compliance and dose related toxicity. The prevalence of cirrhosis is reported to be 10–40%^{4,5} and the prevalence of liver fibrosis is about 40–80%,^{6,7} but these data are over 10 years old. The prevalence of liver disease in TM patients in recent years is not known, and there are no such data from Oriental populations. Since 1980, patients have been given desferrioxamine at a younger age, and so iron overload and its related complications may be less severe. The screening of blood products for hepatitis C antibody has reduced the risk of hepatitis. This study examined the prevalence and severity of liver disease in Chinese TM patients treated in a single institution. The correlation between histological and biochemical markers of iron overload was also examined.

PATIENTS AND METHODS

This was a retrospective study of TM patients treated at the Department of Paediatrics, Prince of Wales Hospital. One hundred TM patients (48 males and 52 females) were included. Forty four had undergone allogeneic bone marrow transplantation (BMT) and 56 patients were receiving regular blood transfusions. Median age at diagnosis was 0.7 years, with 75% less than 1 year. Blood transfusions were administered at 3–4 weekly intervals, to maintain a pretransfusion haemoglobin above 95 g/l. Desferrioxamine was given by subcutaneous infusion over 8–10 hours (20–50 mg/kg), generally 5–6 times weekly. Median age of starting chelation was 3.7 years (range 1.1–18 years). The patients had received chelation therapy for a median of 7.7 years (range 1 month to 22 years). They had received regular transfusions for a median of 10.6 years (range 1 month to 25 years). At time of evaluation, the

median age was 11.7 years (range 1.5–27 years) (table 1). Two patients were positive for hepatitis B surface antigen. Eight were positive for hepatitis C antibody and for hepatitis C RNA.

Liver biopsy

Liver biopsy had been performed in 37 patients prior to BMT between 1992 and 1999. A total of 36 transfusion dependent (TD) patients underwent liver biopsy to assess their liver iron content (LIC). This LIC study was approved by the institution's ethical committee. Informed consent for liver biopsy was obtained from each patient. A total of 81 biopsies were performed on 73 patients. The biopsy specimens were obtained from the right lobe of the liver under ultrasound guidance using a semiautomated 16 gauge Tru-cut style needle (Temno, Bauer Medical International, Dominican Republic). A core of tissue was sent for LIC analysis by graphite furnace atomic absorption.

Histological study

A core of liver tissue was evaluated histologically and graded semiquantitatively for iron storage by a single pathologist. Liver sections were stained with standard haematoxylin and eosin. Iron was shown using the Perls' stain procedure. Grading was based on the method of Scheuler modified by Rowe,⁸ which classified parenchymal and mesenchymal haemosiderin deposition as follows: grade 0, no haemosiderin; grade 1, minimal; grade 2, mild; grade 3, moderate; grade 4, heavy. The presence or absence of fibrosis was recorded. The liver was assessed for evidence of hepatitis.

Biochemical markers

Serum ferritin was measured at six monthly intervals, and the mean serum ferritin in the year before evaluation was recorded. Serum alanine aminotransferase (ALT) levels were

Abbreviations: ALT, alanine aminotransferase; BMT, bone marrow transplantation; LIC, liver iron content; TD, transfusion dependent; TM, thalassaemia major

Table 1 Patient characteristics

	Biopsy (n=73)	Non-biopsy (n=27)	Total (n=100)
Age at diagnosis (y) (Range)	1.1 (1.6) (0–11.2)	1.5 (1.8) (0–8.3)	1.2 (1.6) (0–11.2)
Age started iron chelation (y) (Range)	4.6 (2.7) (1.1–18)	4.4 (3.6) (1.5–16)	4.5 (2.9) (1.1–18)
Age at evaluation (y) (Range)	11.7 (4.5) (1.5–22.5)	10.9 (7.4) (2.7–27)	11.5 (5.4) (1.5–27)
Duration of transfusion (y) (Range)	10.7 (4.9) (0.1–21.5)	9.4 (6.8) (0.7–25)	10.3 (5.5) (0.1–25)
Duration of chelation (y) (Range)	7.4 (3.8) (0.1–19.5)	6.9 (5.3) (0.5–22)	7.3 (4.3) (0.1–22)
Average ferritin (pmol/l) (Range)	5205 (3634) (970–21000)	4727 (2145) (1395–10449)	5078 (3301) (970–21000)
ALT (IU/l) (Range)	38 (45) (9–299)	40 (39) (1–186)	38 (43) (1–299)
LIC* (mg/g dry weight) (Range)	9.6 (8.8) (0.8–41.8)		

Results expressed as mean (SD).

*LIC measured in 43 patients only.

routinely measured prior to monthly transfusion, and levels in the two year period before this evaluation were recorded.

Statistics

Continuous variables were expressed as mean and standard deviation. Comparison of factors potentially associated with haemosiderosis, fibrosis, and elevated serum transaminase was performed using Student's *t* test or analysis of variance as appropriate. These variables included sex, duration of transfusion and chelation, age, serum ferritin, LIC, and hepatitis status. The possible associations between LIC and serum ferritin, and haemosiderosis deposition were explored using Pearson's correlation coefficient or Fisher's exact test. Multivariate analysis by stepwise regression for factors predicting haemosiderosis, hepatic fibrosis, and liver transaminase elevation were performed. The level of significance was set at 0.05 in the analyses and all the statistical testing was two sided.

RESULTS

Histology

All the liver specimens showed at least grade 1 haemosiderosis; grade 3 and 4 occurred in 26% and 18% of the samples

respectively (table 2). Serum ferritin, ALT, and LIC were significantly different among the four grades of haemosiderosis ($p < 0.001$, $p = 0.001$, and $p < 0.001$ respectively). The differences remained significant when grade I and II combined were compared grade III and IV. There was no significant difference in age at evaluation, or in the duration of transfusion and chelation. Multivariate analysis revealed serum ferritin as the most significant predictor of moderate to severe haemosiderosis ($p = 0.003$). However, when multivariate analysis was applied to the 42 in whom LIC was available, LIC appeared to be an even better predictor of moderate to severe haemosiderosis ($p < 0.001$).

Features of hepatitis were reported in seven of the eight patients positive for hepatitis C, but none of these had features of cirrhosis. The two patients positive for hepatitis B surface antigen did not have evidence of hepatitis. Hepatic fibrosis was present in 22 cases and these patients had significantly higher concentrations of serum ferritin, serum ALT, and LIC (table 2). Hepatic fibrosis was more common in those with hepatitis C ($p = 0.008$). Multivariate analysis revealed serum ferritin as the most significant predictor of hepatic fibrosis.

Table 2 Histological changes of liver biopsy specimens and associated factors

		Serum ferritin (pmol/l)	ALT (IU/l)	LIC (mg/g)	Age (y)	Transfusion (y)	Chelation (y)
Haemosiderosis							
Grade I	17 (23%)	2981 (1256)	17 (5)	5.3 (3.0)	10.4 (3.0)	9.3 (3.1)	6.5 (2.5)
Grade II	24 (33%)	4463 (1814)	30 (28)	6.7 (3.8)	11.7 (4.8)	10.8 (4.8)	7.6 (4.1)
Grade III	19 (26%)	6151 (4215)	38 (28)	9.9 (5.4)	13.2 (4.4)	12.8 (4.4)	9.4 (3.2)
Grade IV	13 (18%)	5205 (3634) ($p < 0.001$)	78 (82) ($p = 0.001$)	22.7 (14.3) ($p < 0.001$)	11.4 (5.5) ($p = 0.3$)	9.2 (6.8) ($p = 0.11$)	5.6 (4.6) ($p = 0.03$)
Grade I/II	41 (56%)	3848 (1752)	24 (22)	6.2 (3.5)	11.1 (4.1)	10.2 (4.2)	7.1 (3.5)
Grade III/IV	32 (44%)	6999 (4617) ($p = 0.001$)	54 (59) ($p = 0.01$)	15.5 (11.8) ($p = 0.007$)	12.5 (4.9) ($p = 0.2$)	11.3 (5.7) ($p = 0.34$)	7.9 (4.2) ($p = 0.4$)
Hepatic fibrosis							
Present	22 (30%)	7717 (4193)	72 (67)	13.9 (11.8)	13 (4.8)	11.6 (5.7)	7.8 (5.0)
Absent	51 (70%)	4170 (2824) ($p = 0.001$)	23 (16) ($p = 0.02$)	8.0 (6.9) ($p = 0.048$)	11.2 (4.3) ($p = 0.1$)	10.3 (4.5) ($p = 0.3$)	7.3 (3.2) ($p = 0.66$)

Results expressed as mean (SD).

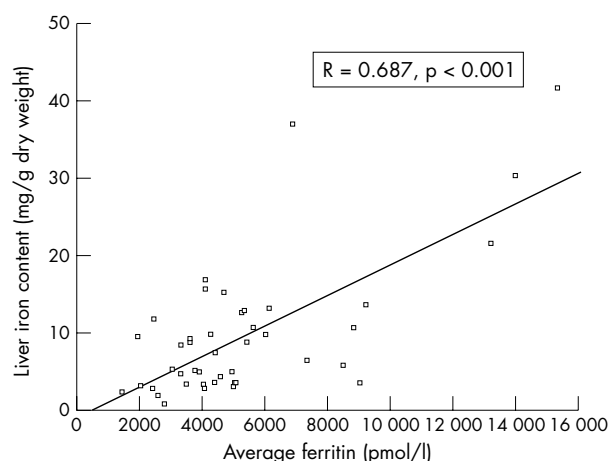


Figure 1 Relation between serum ferritin and liver iron content.

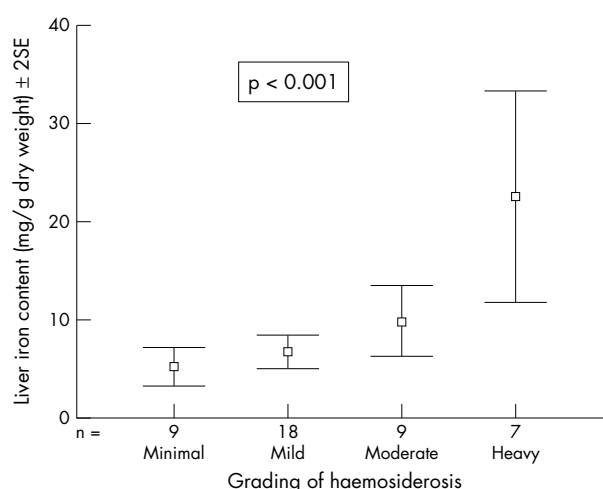


Figure 2 Relation between grading of haemosiderosis and liver iron content.

Liver iron content

Forty three samples were analysed for LIC. The median LIC was 7.5 mg/g dry weight (range 0.8–41.8). Seven samples (16.3%) had an LIC over 15 mg/g, a concentration associated with a high risk for cardiac disease.⁹ The correlation coefficient for LIC and serum ferritin was 0.687 ($p < 0.001$) (fig 1). Considering those with a serum ferritin greater than 5500 pmol/l (2500 µg/l) (a concentration associated with increased mortality¹⁰) the correlation was only 0.46 ($p = 0.11$). There were significant differences in LIC in association with the various grades of haemosiderosis ($p < 0.001$) (fig 2).

Liver enzyme evaluation

There was no correlation between serum ALT concentrations and age, duration of transfusion and chelation, or LIC. Serum ferritin and anti-HCV positivity were the only significant factors associated with increased ALT concentrations (both $p < 0.001$). Eighteen patients (18%) had persistent elevation of ALT and 10 (10%) had transient elevation. The transiently elevated group had significantly higher serum ferritin than the non-elevated group ($p < 0.001$).

DISCUSSION

Despite the use of prenatal screening in Hong Kong for more than 10 years,¹¹ there are still two to five new cases of TM annually. These represent either failures of screening or immigrant children from mainland China. Though the number of

reported deaths from liver disease in TM is small, severe haemosiderosis and hepatic fibrosis may lead to cirrhosis. Treatment is now available for hepatitis C infection.¹² Thus, early and accurate diagnosis of liver disease followed by prompt intervention may prevent liver disease progression. In our study, we found a prevalence of 44% grade 3–4 haemosiderosis; this is similar to that reported approximately 10 years ago.^{5,6} This could reflect the rather unsatisfactory compliance rate with desferrioxamine therapy observed in many of our patients.

Desferrioxamine induced toxicity is relatively common in patients with less severe iron overload.^{13,14} Serum ferritin is an acute phase reactant and may be increased as a result of inflammatory disorders such as hepatitis and ascorbate status. Measurement of LIC is an alternative approach to the assessment of body iron content.¹⁵ This has been studied in both TM and hereditary haemochromatosis patients and is found to be a more accurate indicator of overload.^{9,16} The safety of liver biopsy has been shown in TM patients.¹⁷ The accurate assessment of body iron stores can help guide chelation therapy, and thus reduce desferrioxamine toxicity. Unfortunately, liver biopsy is an invasive procedure, and patients may not accept frequent biopsy as suggested by some authors.¹⁸ Identifying patients who are at high risk of developing fatal complications from iron overload is an important objective. Continuous central venous administration of desferrioxamine may postpone cardiac death.¹⁹

Although there was a correlation between serum ferritin and LIC in this study, this was less reliable at ferritin concentrations above 5500 pmol/l. Patients with “low” serum ferritin can be monitored without biopsy but LIC should be measured in those with a high serum ferritin concentration. Low serum ferritin should alert the physician to the need for desferrioxamine for dose reduction in order to avoid toxicity.²⁰

Liver histological examination provides information about haemosiderosis and hepatic fibrosis. While LIC measurement may not be available in some centres, the grading of haemosiderosis provides a useful measure of iron overload as there was good correlation with LIC. Grade 4 haemosiderosis was always associated with an LIC of more than 10 mg/g dry weight, and such patients require intensive chelation therapy. The presence of hepatic fibrosis points to a risk of progression to cirrhosis.²¹ The pathogenesis of hepatic fibrosis in iron overload is still not well understood, although severe overload is a known risk factor for developing fibrosis. With effective iron chelation, hepatic fibrosis may be reversible. Hepatic fibrosis is also a known prognostic factor for patient survival following BMT, as reported previously.²²

In this study, we did not observe any patients with liver cirrhosis. The 30% prevalence of hepatic fibrosis was lower than that reported previously.^{5,6} In our study, only eight patients were positive for hepatitis C, a much lower prevalence than that reported in Mediterranean TM.²³ This may be a result of the relatively young age of our patients and also the screening of blood products since the early 1990s. The hepatitis C positive patients have entered a trial of combination treatment with interferon and ribavirin.

In conclusion, liver disease is common in TM patients and severe haemochromatosis is still observed in 30–40% of patients despite the use of desferrioxamine chelation. Serum ferritin is useful to monitor treatment in patients who have not yet developed severe iron overload. However patients with high serum ferritin concentrations and those with hepatitis C should be evaluated for LIC, and for hepatic fibrosis and cirrhosis by liver biopsy. In centres where LIC assay is not available, histological grading of haemosiderosis is a useful alternative.

ACKNOWLEDGEMENT

We thank the Hong Kong Children's Thalassaemia Foundation for financial support of the liver iron content study.

.....

Authors' affiliations

C K Li, K W Chik, V Lee, M M K Shing, P M P Yuen, Department of Paediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong
C W K Lam, Department of Chemical Pathology, Prince of Wales Hospital
K F To, Department of Anatomical and Cellular Pathology, Prince of Wales Hospital
S C H Yu, Department of Diagnostic Radiology and Organ Imaging, Prince of Wales Hospital
A Y K Cheung, Centre for Clinical Trial and Epidemiological Research, Prince of Wales Hospital

REFERENCES

- 1 **Li CK**. Current status of thalassaemia major in Hong Kong: 20th anniversary special issue. Hong Kong: Cooley's Anaemia Association, 2001.
- 2 **Borgna-Pignatti C**, Rugolotto S, De Stefano P, *et al*. Survival and disease complications in thalassaemia major. *Ann N Y Acad Sci* 1998;**850**:227–31.
- 3 **Zurlo MG**, De Stefano P, Borgna-Pignatti C, *et al*. Survival and causes of death in thalassaemia major. *Lancet* 1989;**2**:27–30.
- 4 **Jean G**, Terzoli S, Mauri R, *et al*. Cirrhosis associated with multiple transfusions in thalassaemia. *Arch Dis Child* 1984;**59**:67–70.
- 5 **Aldouri MA**, Wonke B, Hoffbrand AV, *et al*. Iron state and hepatic disease in patients with thalassaemia major, treated with long term subcutaneous desferrioxamine. *J Clin Pathol* 1987;**40**:1353–9.
- 6 **Angelucci E**, Baronciani D, Lucarelli G, *et al*. Liver iron overload and liver fibrosis in thalassaemia. *Bone Marrow Transplant* 1993;**12** (suppl 2):29–31.
- 7 **Maurer HS**, Lloyd-Still JD, Ingrisano C, *et al*. A prospective evaluation of iron chelation therapy in children with severe beta-thalassaemia. A six-year study. *Am J Dis Child* 1988;**142**:287–92.
- 8 **Searle JW**, Kerr JFR, Halliday JW, *et al*. Iron storage disease. In: MacSween RNM, Anthony PP, Scheuer PJ, eds. *Pathology of liver*, 2nd edn. Edinburgh: Churchill Livingstone, 1987:185–6.
- 9 **Brittenham GM**, Griffith PM, Nienhuis AW, *et al*. Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassaemia major. *N Engl J Med* 1994;**331**:567–73.
- 10 **Olivieri NF**, Nathan DG, MacMillan JH, *et al*. Survival in medically treated patients with homozygous β -thalassaemia. *N Engl J Med* 1994;**331**:574–8.
- 11 **Ghosh A**, Woo JSK, Wan CW, *et al*. Evaluation of a prenatal screening procedure for β -thalassaemia carriers in a Chinese population based on the mean corpuscular volume (MCV). *Prenat Diagn* 1985;**5**:59–65.
- 12 **Telfer PT**, Garson JA, Whitby K, *et al*. Combination therapy with interferon alpha and ribavirin for chronic hepatitis C virus infection in thalassaemia patients. *Br J Haematol* 1997;**98**:850–5.
- 13 **Olivieri NF**, Buncic JR, Chew E, *et al*. Visual and auditory neurotoxicity in patients receiving subcutaneous deferoxamine infusions. *N Engl J Med* 1986;**314**:869–73.
- 14 **Olivieri NF**, Koren G, Harris J, *et al*. Growth failure and bony changes induced by deferoxamine. *Am J Pediatr Hematol Oncol* 1992;**14**:48–56.
- 15 **Angelucci E**, Brittenham GM, Christine E, *et al*. Hepatic iron concentration and total body iron stores in thalassaemia major. *N Engl J Med* 2000;**343**:327–31.
- 16 **Cartwright GE**, Edwards CQ, Kravitz K, *et al*. Hereditary hemochromatosis: phenotypic expression of the disease. *N Engl J Med* 1979;**301**:175–9.
- 17 **Angelucci E**, Baronciani D, Lucarelli G, *et al*. Needle liver biopsy in thalassaemia: analyses of diagnostic accuracy and safety in 1184 consecutive biopsies. *Br J Haematol* 1995;**89**:757–61.
- 18 **Olivieri NF**, Brittenham GM. Iron-chelating therapy and the treatment of thalassaemia. *Blood* 1997;**89**:739–61.
- 19 **Davis BA**, Porter JB. Long-term outcome of continuous 24-hour deferoxamine infusion via indwelling intravenous catheters in high-risk β -thalassaemia. *Blood* 2000;**95**:1229–36.
- 20 **Porter JB**, Jaswon MS, Huehns ER, *et al*. Desferrioxamine ototoxicity: evaluation of risk factors in thalassaemic patients and guidelines for safe dosage. *Br J Haematol* 1989;**73**:403–9.
- 21 **Gross JB**. Metabolic diseases of the liver. In: Schearman DJC, Finlayson N, Camilleri M, Carter D, eds. *Diseases of the gastrointestinal tract and liver*. Edinburgh: Churchill Livingstone, 1997:951–9.
- 22 **Lucarelli G**, Galimberti M, Polchi P, *et al*. Marrow transplantation in patients with thalassaemia responsive to iron chelation therapy. *N Engl J Med* 1993;**329**:840–4.
- 23 **Lai ME**, De Virgiliis S, Argioli F, *et al*. Evaluation of antibodies to hepatitis C virus in long-term prospective study of posttransfusion hepatitis among thalassaemic children. *J Pediatr Gastroenterol Nutr* 1993;**16**:458–64.

ARCHIVIST.....

Protect the unborn child



Please visit the Archives website [www.archdischild.com] for link to this full article.

HIV therapy and folate antagonists in the first trimester can lead to birth defects, a UK study has found. With HIV increasing among women, more are taking antiretroviral therapy (ART) and other drugs with the potential for causing birth defects to control HIV associated conditions. No excess risk of birth defects arises from ART, according to the International Antiviral Pregnancy Agency, but no data exist for use with other extensively used drugs like folate antagonists.

Jungmann *et al* looked for links between use of ART and folate antagonists in the first trimester and birth defects in a retrospective case note study of mother-infant pairs. The mothers, known to be HIV positive beforehand, delivered at one of six inner London hospitals from May 1994 to June 1999.

Among 195 mother-infant pairs ART was used increasingly in the first trimester (0% to 28%, 1994 versus 1998). Forty four (23%) infants were exposed to other drugs in the first trimester. Nine infants (4.6%) had birth defects. Infants exposed to both ART and folate antagonists during the first trimester had seven times the risk of birth defects of those exposed to neither 23.1% (3/13) versus 4% (6/148); odds ratio 7.10, 95% confidence interval 1.5 to 34.2). None of 34 infants exposed to ART or folate antagonists alone in the first trimester had birth defects.

Excluding maternal characteristics as possible confounders, the authors concede their study's other limitations. Still, they say, doctors should keep reviewing the need for prophylactic folate antagonists in women of childbearing age.

▲ *Sexually Transmitted Infections* 2001;**77**:441–3.